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14. ABSTRACT Mesenchymal stem cells (MSCs) have been proposed to be cellular vehicles for the targeted delivery and local production of biological agents in tumors. In this proposal we will stably transfect mesenchymal stem cells with a lentiviral vector containing a therapeutic gene and dual reporter gene mrfp-ttk. Specific Aims: 1) We will monitor breast cancer tropism of mesenchymal stem cells by multimodality imaging techniques; 2) We will demonstrate the ability of mesenchymal stem cells to target delivery of gene therapeutics to breast cancer in vitro; 3) We will determine the effect of mesenchymal stem cell to target delivery of gene therapeutics to breast cancer lung metastasis. Major findings from year 1 studies: 1) MSCs home to both subcutaneous breast cancer and its lung metastasis; 2) MSCs home to both premature and well-established breast cancer lung metastasis; 3) MSCs proliferate at tumor site; 4) MSCs show dissimilar differentiation potential at lung tumor and subcutaneous tumor niches; 5) lung tumor microenvironment upregulates BMP-2 and Noggin transcription, which favor MSCs osteoblastogenic differentiation.					
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INTRODUCTION

In our previous annual report, we found that mesenchymal stem cells (MSCs) homing to breast cancer lung metastasis differentiate into osteoblasts, whereas MSCs homing to subcutaneous breast cancer differentiate into adipocytes. The data provide evidence that MSCs home and engraft to tumor site, and that different tissue tumor niche have distinct effect on MSCs differentiation. These finding may be clinically relevant because the beneficial effects of MSCs are being tested clinically in attempts to improve hematopoietic engraftment [1], to treat osteogenesis imperfect [2], graft-versus-host disease [3], and autoimmune diseases [4, 5], and to deliver therapy for malignancies [6, 7].

For the current funding period, we have focused on our specific aim II presented in the research proposal: We will demonstrate the ability of mesenchymal stem cells to target delivery of RGD4CrmhTNF fusion protein to breast cancer cells and endothelial cells in vitro.

BODY

To achieve our goal, we obtained the plasmid RGD-rmhTNF (recombinant mutated human TNF) from our collaborator, Dr. Yinqi Zhang, MD, PhD, Professor and Director of Center of Biotechnology at The Fourth Military Medical University in Xian, China. Then we finished the design of vector and our cloning strategy is listed below. We are actively working on the cloning the vector and expect to obtain the vector very soon.

The lentivirus vector, pLVX-IRES-ZsGreen1 from Clontech, will be used to stably express the fusion protein of rmhTNF-mrfp-ttk in stem cells. First, the restriction site of AgeI will be inserted upstream of ZsGreen1 sequence by using GeneTailor Site-Directed Mutagenesis system from Invitrogen. Also, a PacI site will be added at the 3' end of ZsGreen1 sequence by using the same strategy. The fragment of RGD-rmhTNF (recombinant mutated human TNF) was amplified from PBV220-RGD-rmhTNF by using the sense

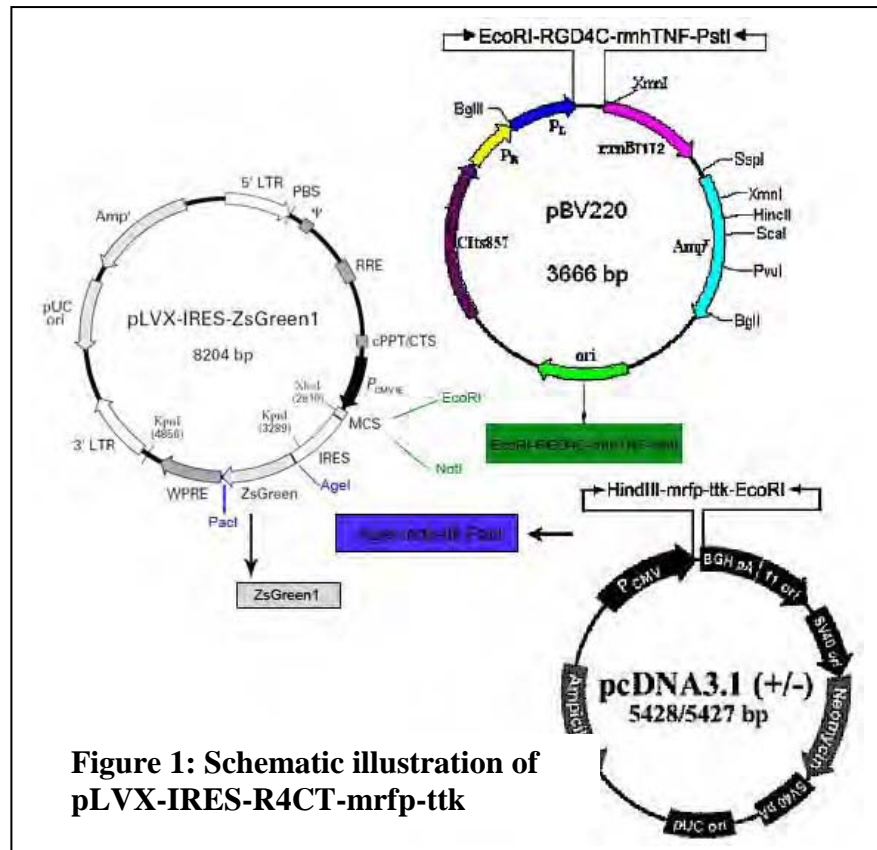
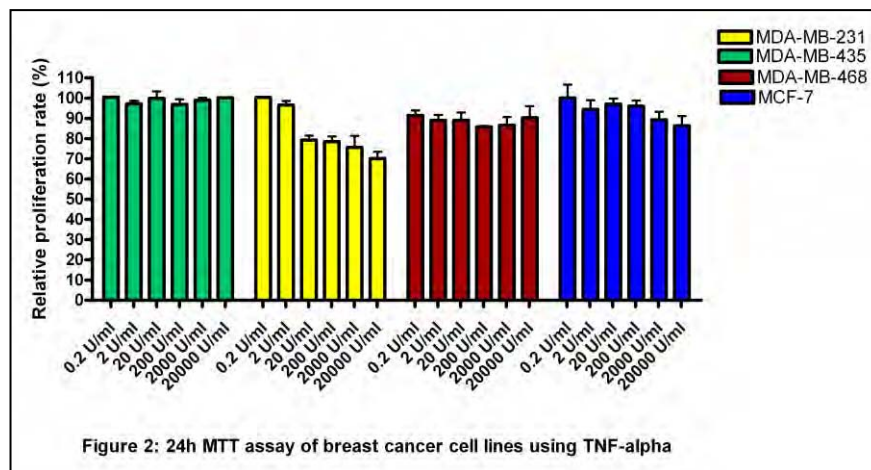


Figure 1: Schematic illustration of pLVX-IRES-R4CT-mrfp-ttk

primer of 5'-GCGAATTC ATGCGTCGTCGTTCTCTTCTTATTCTTGTTATGTGC-3' and the antisense primer of 5'- GGAGCGGCCCGCTCAGAATTCAATGA-3'. The restriction site of EcoRI and NotI underlined in the primer sequences were added respectively to the 5' and 3' end by PCR. The italicized sequence, ATGCGTCGTCGTTCTCTTCTTATTCTTGTT, encodes the signal peptide of MRRRSLLILV. Both PCR product and the modified vector of pLVX-IRES-ZsGreen1 (pLVX-IRES-A-ZsGreen1-P) will be digested with EcoRI and NotI and ligated by T4 ligase. The fragment of mrfp-ttk were amplified from the vector of pcDNA3-hrl-mrfp-ttk (a kind gift from Dr. Gambhir at Stanford University) by using the primer set of 5'-GCACCGGTATGGCCTCCTCCGAGGAC-3' and 5'-ACGCTTAATTAA TCAGTTAGCCTCCCC-3' with AgeI added to the 5' end and PacI inserted to the 3' end, both sites were underlined in the primer sequences. Both PCR product and pLVX-IRES-A-ZsGreen1-P will be digested with AgeI and PacI. The fragment of mrfp-ttk will then be inserted downstream of IRES and replace ZsGreen1 sequence.

In the meanwhile, to choose a TNF sensitive breast cancer cell line, we did 24h MTT assay using several breast cancer cell lines and found MDA-MB-231 is the most sensitive breast cancer cell line among others such as: MDA-MB-435, MDA-MB-468 and MCF-7. Figure 2 is the result of MTT assay.

Based on the MTT assay, we will select MDA-MB-231 cells for in vitro study and for preparation of lung metastasis tumor model. To monitor metastasis tumor growth, we need to transfect reporter gene of firefly luciferase into cells in order to apply bioluminescence imaging. We already did the transfection and cells are currently in the process of antibiotic screening for obtaining stable cell lines.



KEY RESEARCH ACCOMPLISHMENTS

There is no key research accomplishment in this reporting period.

REPORTABLE OUTCOMES

Because of limited progress of the project, there have been no manuscripts, abstracts, or products, patents, and career developments that resulted from this award during the reporting year.

CONCLUSIONS

In conclusion, because of funding transferring process, we lost quite significant amount of research time. The pLVX-IRES-R4CT-mrfp-ttk vector has been under cloning.

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APPENDICES

None.